

REMARKS

Examiner's Amendment Summary

On 7 October 2003, Applicants' attorney carried out an Interview with Examiner Smith and Supervising Examiner Marschel concerning certain aspects the rejection and strategies for overcoming the rejection. Applicants' attorney had raised concerns over the rejections characterization of the tag-polymerizing agent interaction as being transient, because the claim language was amended to expressly recite that the tag-polymerization agent interaction was a covalent bond. Applicants' attorney stated that he knew of no more "permanent" attachment format than a covalent bond, while recognizing that all bonds are breakable. Mr. Marschel then articulated that it was more the fact that the claim wording could cover other type of events that would cause the tag to undergo a change in a detectable property that concerned the Patent Office on claim scope. It was then suggested that the addition of language to indicate that the tagged polymerizing agent was to be used in a sequencing reaction may serve to center the claims within the context of the subject matter of the specification. The language may also simultaneously clarify and distinguish the claims over other types of reactions that may also cause a change in the detectable tag property such as attachment of a transition state analog to the polymerization agent. Applicants' attorney agreed to respond to the Office Action with a set of amended claims with language designed to clarify and distinguish the claims over the cited prior art and to act as a vehicle for a potential second Examiner's interview.

DETAILED ACTION AND RESPONSE

Applicants acknowledge that the Examiner stated:

Applicants' amendments and remarks in Paper No. 16 and 20, filed 4/3/03 and 6/20/03, are acknowledged. Amended claims 1, 7, 10, 16, and 20 are acknowledged. The species election requirement is hereby withdrawn as generic claim 10 appears to be free of the prior art.

Applicants acknowledge that the Examiner stated:

Applicants' arguments, filed 4/3/03, have been fully considered but they are not deemed to be persuasive. Rejections and/or objections not reiterated from the previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the set presently being applied to the instant application.

Applicants acknowledge that the Examiner stated:

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR § 821 (a)(1) and (a)(2). However, this with the requirements of 37 CFR § 1.821 through

1.825, because many, if not all, of the newly added SEQ ID NOs (to the specification) do not correspond to the sequences in the Sequence Listing, particularly SEQ ID NO: 11. Applicant(s) are required to submit a new computer readable form sequence listing, a paper copy, or CD-ROM for the specification, statements under 37 CFR §1.821(f) and (g), if there is a need to list additional sequences in the sequence listing: Applicant(s) are given the same response time regarding this failure to comply as that set forth to respond to this office action. Failure to respond to this requirement may result in abandonment of the instant application or a notice of a failure to respond to this Office Action.

Applicants will submit a new sequence listing to conform with the sequence requirements.

Applicants acknowledge that the Examiner stated:

The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed. The present title is directed to real-time sequence determination a method-type invention whereas in contrast the elected claims include a composition of the polymerizing agent type.

Applicants have amended to the title to be commensurate with the subject matter now being claimed.

Applicants acknowledge that Claims 1-24 are herein under examination.

Claim Rejections 35 U.S.C. § 112, First Paragraph

Claims 10-19 stand rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for an interaction of tags relating to acceptor and donor interactions, does not reasonably provide enablement for other types of interactions.

The Examiner contends as follows:

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. The specification provides enablement for detecting extension products containing a fluorescent tag (p. 3, line 9), using dye-terminator chemistry with donor and acceptor dyes (p.3, lines 21-32), including a donor-acceptor pairs (p. 20, lines 20-23). The breadth of the claims encompasses a broader set of interactions which is not sufficiently enabled in the specification.

Applicants disagree with the Examiner's assessment of the type of tags and the nature of the properties than can be detected by the tags. The specification clearly discloses the use of labels or tags that are NMR active, UV active, far UV active, IR active or fluorescently active. The use of such optically or magnetically active tags is well known in the art and any ordinary artisan would understand how the analytical system would operate to obtain measurements of the compositions of this invention. However, applicants have decided to focus the application on fluorescently active

tags or tag pairs so narrow the focus of the examination, while still maintaining their position that other tags and their corresponding detection systems can be used as well such as nmr spectrometry, IR spectrometry and UV spectrometry.

Claims Rejected Under 35 U.S.C. § 112, Second Paragraph

The rejection of claims 6, 16, 18-19, and 24 is maintained under 35U.S.C.112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

The Examiner contends as follows:

Claims 6 (line 3), 19 (line 3), and 24 (line 3) recite the phrase "mixtures or combinations thereof of the *Taq* polymerase" which is vague and indefinite. It is unclear what else may be included in these multiple entities besides *Taq* DNA polymerase I. Clarification of the metes and bounds of these claims via clearer claim wording is required.

Applicants has amended claim 6 to remove the unclear language. The language was intended to cover the case where the polymerizing agent or polymerase had two or more molecular tags. But has been removed to clarify the claim. This clarifying language does not narrow the claim because the amino acid sites

The Examiner contends as follows:

Claim 10 is rejected due to the lack of clear antecedent basis for "the polymerase" and the polymerizing agent, on line 5. The claim begins by stating "a polymerizing agent" to include both "the polymerase" and a monomer. The last mention of "polymerizing agent" is separate from the monomer. Clarification of these discrepancies via clearer claim wording is required. Claims 11-19 are also rejected due to their direct or dependency from claim 10.

Applicants has amended the claim to make the language consistent. Of course, this amendment does not narrow the scope of the claim in that only the reference element was made consistent.

The Examiner contends as follows:

Claim 16 is vague and indefinite due to the unclarity of citing an abbreviation, such as dNTP on line 1. Correction is suggested by amending in of the full name in parentheses. Claim 18 is also rejected due to its dependency from claim 16.

Applicants have amended claim 16 to include the long name for dNTP. Again, this is a non-narrowing amendment because it only includes the definition of the term dNTP.

The Examiner contends as follows:

Claim 16, line 2, recites the term "group" which is vague and indefinite. It is unclear which entity the "group" is a part of, the polymerase, the monomer, or the dNTP. Clarification of the location of the "group" is required. Claim 18 is also rejected due to its dependency from Claim 16.

Applicants have amended the claims to make clear that the tag is bonded to the beta or gamma phosphate of the triphosphate of the dNTP. Again, this is a non-narrowing amendment because the beta and gamma site are only associated with the triphosphate.

Claim Rejections - 35 U.S.C. § 102

Claims 1-5, 7-9, and 20-23 stand rejected under 35 U.S.C. § 102(a) and (b) as being anticipated by Williams (WO 00/36151 under 102(a)) and Brandis (Nucleic Acids Research, 1999, Vol. 27, No. 8 under 102(b)).

The Examiner contends as follows:

Applicants state the critical difference between their invention and the prior art is that the tags in their invention remain associated with the polymerizing agent. This is found unpersuasive as the claims, as written, state a polymerizing agent and a tag without mention of whether this tag is transient or permanent. Applicants state the Williams/Brandis prior art references do not disclose tagged polymerizing agent. This is found unpersuasive as bonding inherently occurs between the polymerase and the tagged entity, as will be further described, infra. Even though this bonding may occur transiently, it does occur which is encompassed in the broad reasonable interpretation of the tagged polymerase agent.

Williams discloses a *Taq* DNA polymerase (p. 8, lines 23-28) in which a fluorescently labeled dNTP (tag) is associated with the polymerase during monomer incorporation (p. 8, lines 1-9). Williams discloses a fluorophore and quencher pair being incorporated into oligo probes (p. 2, lines 16-18). The dNTP tag consists of a labeled nucleotide triphosphate (NTP) having a γ -phosphate with a fluorophore moiety attached and a quencher moiety that sufficiently prevents fluorescence until incorporation of the NTP at which time the γ -phosphate with the fluorophore moiety is released and detected (p. 8, lines 10-20). As Webster's II New Riverside Dictionary defines a tag as a piece of something that identifies, classifies or labels; one reasonable interpretation of the quencher is a tag whose close presence to the fluorophore tag results in fluorescent signal disappearance (p. 2, lines 16-25). Williams discloses the fluorescence is detected when labeled dNTPs are incorporated into the strand and fluorescence is induced (p. 9, lines 28-29). Williams discloses that upon incorporation, the fluorescent dye molecule is released with pyrophosphate from the polymerase and then swept away from the parent DNA molecule by the flow (p. 10, lines 13,-17), suggesting the polymerase's detectable property reverts back to its initial state. Williams discloses that as the polymerase moves along the DNA, the nucleotide sequence is read from the order of released dyes (p. 41, lines 30-31). Williams discloses the possible presence of other polymerases, such as HIV reverse transcriptase, as stated in Claims 5, 9 ,and 23.

A 35 U.S.C. 102 rejection over multiple references has been held to be proper when the extra references are cited to show that a characteristic not disclosed in the reference is inherent (see MPEP2.131.01(c)). Brandis discloses a *Taq* DNA polymerase I including an inherent characteristic that polymerases go through conformational changes (abstract). Brandis also discloses that a change occurs in a fluorescent label during the change in conformational states of the polymerase when

nucleotide binding occurs as the polymerase is active (abstract). Voet et al. disclose a transition state theory allowing the understanding of how enzymes catalyze reactions (p. 332, col 2; first paragraph). Voet et al. disclose a high-energy (unstable) complex existing with covalent bonds during a bimolecular reaction (p. 332, col. 2, second paragraph).

Thus, Williams and Brandis anticipate claims 1-5, 5-9 and 20-23 of the instant invention

Applicants has amended the claims to clearly state that the tag is a fluorescent tag covalently bonded to the polymerizing agent and the fluorescent tag undergoes a change during each of a sequence of monomer incorporations producing data evidencing a read out of the incorporated events and the identity of the NTPs. The tag on the polymerizing agent is not a substrate transiently held within the active site of the polymerizing agent during monomer incorporation, but is an atomic or molecular species that is part of the polymerizing agent (or part of a molecule associated with the polymerizing agent such as a co-factor, a part of the invention that is not being considered at this time).

On the contrary, Williams/Brandis discloses a detection method based on dNTPs having both a quencher and a fluorescent tag covalently bonded thereto in such a way that either the quencher or tag leaves with the released pyrophosphate. The incorporation transiently places the incorporating monomer into the active site within the polymerizing agent. The incorporation event breaks the triphosphate bond of the dNTP supplying the energy to add the next nucleotide to the growing nucleotide sequence releasing the pyrophosphate and dequenching the fluorophore. There is no suggestion to covalently bond a tag to the polymerizing agent itself. In fact, such a structure would not work in Williams/Brandis process because irrespective of which tag you decided to attach to the polymerizing agent, you render the Williams/Brandis process inoperable. Both tags are required to be bonded to the dNTPs for the Williams/Brandis process to be operable.

Regardless of whether a polymerizing agent undergoes a change in conformation during each monomer incorporation event, as is well known in the art, the incorporation event is monitored by a change in the fluorescent property of the tag when the polymerizing agent catalyzes an incorporation event. In the present invention, the tag on the polymerase evidences each incorporation event by undergoing a detectable change of the tag's fluorescent property either directly due to environment changes or due to interaction with a second tag either on the polymerizing agent itself or on the dNPT. Moreover, unlike Williams/Brandis process, the present invention generates natural sequences a not modified sequences (no base labeling).

Because Williams/Brandis do not disclose polymerase having a covalently bound fluorescent

tag that is directly involved in monitoring dNTP incorporation, Williams/Brandis cannot anticipate the present invention as claimed in claims 1-5, 7-9 and 20-23. Furthermore, Williams/Brandis do not render the present invention obvious because Williams/Brandis do not disclose, teach or suggest chemically bonding a tag to the polymerizing agent, which would render the Williams/Brandis process wholly inoperable. Applicants, therefore, respectfully request withdrawal of this section 102 rejection.

Claims 1, 3, and 4 stand rejected under 35U.S.C. 102(e)(2) as being anticipated by Patel et al. (P/N 6,329,178).

The Examiner contends as follows:

Applicants state Patel et al. do not disclose tagged polymerases that undergo a change during monomer incorporation. This is found unpersuasive as claims 1, 3, and 4 are directed to a composition of a polymerizing agent including a tag which has detectable properties that can undergo a change. Covalent bonding is an inherent characteristic that occurs during monomer incorporation (as further described, infra). During this bonding process, the polymerases are attached to the tags, albeit transiently. Applicants cite other instances, such as certain regions of the polymerase and using cysteine as the mutant amino acid, as major differences between the Patel et al. reference and the instant invention. This is found unpersuasive as these limitations are not addressed in the instantly rejected claims 1, 3, and 4.

Patel et al. disclose the Taq DNA polymerase I (col 3, lines 5-8) active site is highly mutable and can accommodate many amino acid substitutions without significantly affecting activity (col. 2, lines 63-66). Patel et al. disclose that mutant DNA polymerases can incorporate unconventional nucleotides (col. 3, lines 44-48), such as bases labeled with a reporter molecule and fluorescently labeled bases (col. 6, lines 14-19) which suggests types of tags. Patel et al. disclose the fluorescently labeled tags exhibiting different emissions when a DNA fragment is extended by DNA polymerase (col. 10, lines 38-67). Patel et al. disclose a polymerase having enzymatic properties featuring catalytic activities (col. 5, lines 1-20).

A 35 U.S.C. 102 rejection over multiple references has been held to be proper when the extra references are cited to show that the characteristic not disclosed in the reference is inherent (see MPEP 2131.01(c)). Voet et al. disclose a transition state theory allowing the understanding of how enzymes catalyze reactions (p 332, col. 2, first paragraph). Voet et al. disclose a high-energy (unstable) complex existing with covalent bonds during a bimolecular reaction (p 332, col. 2, second paragraph).

Thus, Patel et al. anticipates claims 1, 3, and 4.

Applicants respectfully disagree with the Examiner's position on this matter and the idea of covalently bonding. At no time during a DNA monomer incorporation event (template extension) is the dNTP covalently bonded to the polymerase. In fact, no part of the primer, template or

monomer is covalently bonded to the polymerase during template extension. The polymerase merely holds the pieces in an orientation to facilitate 3' nucleophilic attack of the primer onto to bound monomer at its alpha phosphate to liberate pyrophosphate. Although the three pieces necessary for primer extension are clearly held in place by the polymerase, at no time are any of them "covalently bonded" to the polymerase.

Patel et al. disclose mutant polymerases including *Taq* DNA polymerase I and further that the mutants have different incorporation fidelities for naturally occurring dNTPS, rNTPs or modified dNPTs. However, Patel et al. does not disclose, teach or even suggest covalently bonding a tag to the polymerase itself. Moreover, Patel et al. is not concerned with sequencing DNA, but in producing polymerases that have altered incorporation fidelities.

The present invention relates directly to producing an incorporation event read out and the identity of each incorporated monomer. Patel et al. is only involved in producing polymerases with alter incorporation fidelities and not with obtaining a read out of incorporation events and base identities. It is the tag on the polymerase that provides a critical component of the sequencing composition of this invention. Without the tag on the polymerase, sequencing of data corresponding to a template would not occur as claimed.

Simply stated, the Patel et al. mutant polymerases are not the same as the polymerases of this invention. The Patel et al. mutants do not include tags on the polymerase designed to provide incorporation data evidencing monomer incorporation events and base identity to obtain sequencing information.

Because Patel et al. do not disclose tagged polymerases, Patel et al. cannot anticipate the claims 1, 3, and 4. Applicants, therefore, respectfully request withdrawal of this 102 rejection.

Moreover, Patel et al. cannot render the present invention obvious because Patel et al. do not suggest sequencing using a composition including a tagged polymerase, where a property of the tag undergoes a change during an incorporation cycle producing data evidencing the incorporation cycle and the identity of the monomer incorporated to generate real time sequence information.

Claims 1, 2, 3, and 7 stand rejected under 35 U.S.C. 102(e)(2) as being anticipated by Allen (P/N 6,280,939).

The Examiner contends as follows:

Allen discloses a polymerase that produces motions detectable during monomer incorporation (abstract). These motions are caused while the polymerase incorporates nucleotides into a chain and the newly formed nucleotide strand translocates through the polymerase's reaction site(abstract). Allen discloses

detecting the second conformational changes in motion (data collection) after the DNA/polymerase complex has formed and the polymerization temperature is reached (col. 15, lines 26-42). The continuous stream of reaction data as represented in Figures 3 and 5(note the detection peaks as incorporation occurs in the top 4 rows or Figure 3) [as well as col. 4, lines 28-45], as one nucleotide is incorporated at a time shows that changes from first state and first value second state and value and back to first state and value before, during, and/or after monomer incorporation as stated 1 and 2. Allen discloses placing an atomic force microscopic probe tip placed at a polynucleotide/polymerase complex to detect the polymerase motion changes (col. 4, lines 3-14). Allen discloses the DNA template rests in the polymerase reaction site in the polynucleotide/polymerase complex (col. 5, lines 49-54). Allen discloses conformational changes of the polymerase structure in the complex that occur during the polymerase reaction (col. 6, lines 45-65) which represent a type of molecular tag indicating change based on motion detection.

A 35U.S.C. 102 rejection over multiple reference has been held to be proper when extra references are cited to show that the characteristic not disclosed in the reference is inherent (see MPEP 2131.01(c)). Voet et al. disclose a transition state theory allowing the understanding of how enzymes catalyze reactions (p. 332, col. 2, first paragraph). Voet et al. disclose a high-energy (unstable) complex existing with covalent bonds during a bimolecular reaction (p. 332, col.2, second paragraph).

Thus, Allen anticipates the limitations in claims 1, 2, 3 and 7.

Applicants believes that the Examiner is of the opinion that the claims read on a compositions that includes a polymerase, a primer, a template and a monomer to be incorporated. Clearly, the present invention does not relate to this system as it has been used in the public domain for year. The present invention relates to a polymerase that includes a chemically bonded - "covalently bonded" – atom and/or molecular tag having a detectable property. Although the Applicants now have elected to focus on fluorescent properties (the specification is enabling for nmr and other detectable properties), the sequencing composition of this invention requires a tagged polymerase, where the incorporation events are detected by monitoring the detectable property of the tag on the polymerase. This tag is designed to remain bonded to the polymerase via the strongest known chemical interaction, a covalent bond. It if only through the monitoring of the detectable property during each monomer incorporation cycle (approach, binding, incorporation and translocation) that data evidencing each incorporation cycle and the identity of the base sequence incorporated is determined. The claims have amended to made this point as clear as possible.

On the contrary, Allen does not monitor a change in a property of the polymerase or anything associated with the polymerase, but instead looks at a change AFM tip separation from the polymerase complex, which changes due to the known conformational change of the polymerase or the complex during each monomer incorporation cycle. Like Patel et al., the Allen composition

(AFM tip, polymerase, primer, template, and monomer) can utilize flagged (assumed to be the same as tagged) dNTPs, but the flag is designed to change the polymerase incorporation cycle time which in turn changes the AFM response to associated with each incorporation cycle.

Allen does not disclose monitoring a property of a tag covalently bonded to the polymerase to detect polymerase activity, but discloses a system including a polymerase and an AFM tip, where the property of the AFM tip is used to monitor polymerase activity. This is not anticipation of the present system which requires a tag covalently bonded to the polymerase, where a detectable property of the tag is monitored to evidence monomer incorporation and to determine monomer sequences.. Applicants, therefore, respectfully request withdrawal of this 102 rejection.

Moreover, Allen cannot render the present invention obvious because Allen does not suggest sequencing using a composition including a tagged polymerase, where a property of the tag undergoes a change during an incorporation cycle producing data evidencing the incorporation cycle and the identity of the monomer incorporated to generate real time sequence information.

Finally, any combination of these references also does not render the present invention obvious, because none of the references disclose covalently bonding an atomic and/or molecular tag to a polymerase, where the polymerase tag is directly involved in sequence determination either by monitoring changes in a property of the tag on the polymerase directly or monitoring changes in the property due to its interaction with a second tag either on the polymerase or on the monomer.

New Claims

Concerning new claims 35 through 46, the Examiner is authorized to charge any additional claim fees to deposit account no. 501518.

Claims 35-42 and 46 relate to a composition that includes a fluorescent donor tagged polymerase and fluorescent acceptor tagged dNTPs, where excitation light causes a FRET response from each incorporating monomer and the FRET response produces an incorporation event read out and a sequence read out when the different tags or unique tags are used. This composition is not disclosed by any of the reference either individually or in any combination. Applicants, therefore, believe that the new claims are allowable over the prior art.

Applicants, therefore, respectfully request that the amended claims be passed onto allowance.

If it would be of assistance in resolving any issues in this application, the Examiner is kindly invited to contact applicant's attorney Robert W. Strozier at 713.977.7000

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Respectfully submitted,

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